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# The Cobalt(III), Chromium(III), Copper(II), and Manganese(III) Complexes of Ethylenebis((o-hydroxyphenyl)glycine): Models for Metallotransferrins

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The Co(III), Cr(III), Mn(III), and Cu(II) complexes of ethylenebis((o-hydroxyphenyl)glycine) have been prepared as models for the metal binding site in the iron transport protein transferrin. The meso and racemic forms of the ligand have been successfully separated and the metal complexes of each isomer examined, allowing the examination of steric effects on physical properties. Optical, electron spin resonance, and nuclear magnetic resonance spectroscopies have been used to characterize these complexes and to compare them to the appropriate transferrin spectra. The results presented here and elsewhere indicate that EHPG is a reasonable model for the metal binding in transferrin.

## Introduction

Serum transferrin is a glycoprotein of 80 000 molecular weight that is found in the blood of mammals.[1] This protein, which tightly but reversibly binds two ferric ions, is responsible for the transport of this vital element from sites of absorption to sites of utilization and storage. It may also have several other roles of clinical importance: functioning to "buffer" metal concentrations in the blood and acting as an antibacterial agent.[2–4]

Although serum transferrin and related proteins have been studied extensively, many questions concerning their structure and function remain. Of these perhaps the most interesting to inorganic chemists is the nature, including coordination geometry and stereochemistry, of the metal binding sites. It appears that substantial information in this regard could be gained by a study of model compounds that closely resemble what is known about the metal sites.

Recently we have begun the evaluation of several metal chelates as models for the binding site in serum transferrin.[5–7] Certainly the most studied of these is the iron complex of ethylenebis((o-hydroxyphenyl)glycine), FeEHPG. The ligand itself contains the same types of donor groups previously implicated in the protein.[1] The iron complex has been recognized as a potential model for diferric transferrin originally on the basis of its optical and resonance Raman spectra and more recently on the basis of its EPR and solution chemistry.[7,8] Although the iron complex does indeed share many similarities with the native protein, we have sought a more rigorous test and so have extended this comparison to include other metal EHPG complexes and their corresponding metallotransferrins.

## Experimental Section

**Materials.** The ligand (EHPG) was purchased from Sigma Chemical Co. and purified as described by Pecoraro et al.[5] The ligand so obtained consists of an unspecified mixture of meso and racemic isomers. For some of the studies described herein we have examined these isomers separately. The pure meso ligand was prepared by removing the iron from *meso*-FeEHPG prepared as previously described.[7] The iron was removed by reduction with a large excess of sodium dithionite at pH pH ~3–4 in acetate buffer. The zwitterionic ligand was filtered off and washed thoroughly with water and methanol. Its identity was confirmed by NMR.

Pure racemic ligand was prepared by acidification of a solution of NH₄[VO(EHPG)]·H₂O·C₂H₅OH to a pH of 2.5.[9] Once again the ligand was filtered off and washed with water and acetone. NMR served to establish its identity.

**Preparation of Complexes.** Metal complexes were prepared as previously described in the case of the *rac* Co(III) and *rac* Cu(II) complexes.[6]

*rac*-Na[Mn(EHPG)]·1.5H₂O. Mn<sup>III</sup>EHPG was synthesized by reacting 8 mmol of EHPG, in degassed H₂O, to which 2 equiv of NaOH was added under N₂ pressure, with 8 mmol of manganese(III) acetate. The dark brown solution was allowed to stir overnight. Alternatively, MnCl₂ could be used as the manganese source but the reacting solution was allowed to stir open to the air overnight to effect the oxidation of the Mn(II) to Mn(III). The solution was filtered and evaporated to dryness. The product was recrystallized from 70% EtOH/H₂O. Due to the highly oxidizing nature of Mn(III) the yield of purified products was always low. Extensive recrystallization usually leads to the formation of a highly crystalline, but nearly insoluble, unknown material which contains no Mn and, we believe, is oxidized ligand. Mn was determined as permanganate as described by Vogel.[10]

Anal. Calcd for Na[Mn(EHPG)]·1.5H₂O: C, 46.8; H, 4.1; N, 6.1; Mn, 11.9. Found: C, 46.3; H, 4.03; N, 5.7; Mn, 12.2.

*rac*-K[Cr(EHPG)]·3H₂O. The chromium(III) complex, an unspecified isomer of which has previously been reported,[11] was prepared by reacting EHPG with 2 equiv of KOH in water and adding a stoichiometric quantity of CrCl₃·6H₂O. The mixture, which gradually turned pink-violet, was allowed to stir from several days to 1 week. The reaction could also be run in considerably less time by using chromous acetate as the chromium source. The solution was filtered and evaporated to dryness. The product apparently consisted of two compounds, one of which had an $R_f$ on TLC identical with that of *rac*-FeEHPG, with a smaller amount having a lower $R_f$. Extensive recrystallization with water and water/EtOH mixtures gave red crystals of *rac*-K[Cr(EHPG)]·3H₂O.

Anal. Calcd for K[Cr(EHPG)]·3H₂O: C, 43.0; H, 4.4; N, 5.5. Found: C, 43.1; H, 3.4; N, 5.5.

The following complexes of the pure meso ligand were prepared in solution only, and no attempt was made to isolate analytically pure solids. *meso*-CoEHPG was prepared for solution studies by the following procedure. CoCl₂ was mixed with *meso*-EHPG in 10 mL of 1.5 M NH₄OH. A dark brown color developed. The solution was stirred in the air for 3 days. A small amount of 3% H₂O₂ was added to oxidize any Co(II) to Co(III). The product was filtered and rotovaped to dryness. It was taken up in methanol and adsorbed on a layer of silica gel and eluted with more methanol. It was rotovaped to dryness again. TLC demonstrated that the product was mainly the desired *meso*-CoEHPG but was still impure. Final purification was achieved via chromatography on Bio-Gel P-4 (Bio-Rad) with water

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**Table I.** NMR Parameters for EHPG in Alkaline $D_2O$

| ligand (EHPG) isomer | assignt | | |
|---|---|---|---|
| | α-H | NCH₂CH₂N | phenyl |
| racemic | 4.19 (s) | 2.63 (m) | 6.99 (m), 6.64 (m) |
| meso | 4.25 (s) | 2.63 (m) | 6.99 (m), 6.61 (m) |
| commercial | 4.19 (s) 4.25 (s) | 2.64 (m) | 6.99 (m), 6.63 (m) |

as an eluant. A yellow band remained at the origin, and a dark red band and a light red band were eluted. The dark red band was collected and proved to be pure *meso*-CoEHPG as determined by TLC and NMR.

The meso $Cu^{II}$, $Mn^{III}$, and $Cr^{III}$ EHPG complexes were prepared in an analogous manner.

**Thin-Layer Chromatography.** Purity of products and the presence of isomers were assayed via TLC on Silica-Gel, G-60 (Merek) pre-coated glass plates using the upper layer of 1-butanol/$H_2O$/acetic acid (4:5:1) as an eluant. The isomers, meso and *rac*, of FeEHPG ($R_f$ = 0.33 and $R_f$ = 0.29, respectively) were used as standards. Spots were detected by visual means or by staining with $I_2$.

**Physical Measurements.** Metal complex NMR spectra were recorded in $D_2O$ solution on a Bruker 250 MHz NMR spectrometer. Free-ligand spectra were run in alkaline $D_2O$ ("pD" adjusted with NaOD). All chemical shifts are recorded vs. HDO (4.60 ppm). The spectra were analyzed with use of the program UEANMR of Jahannesen and Ferretti as modified by S. Fleischman for the University of Vermont Dec computer.

UV–vis spectra recorded in water on a P&E 552 spectrophotometer. Calculation of ligand field parameters followed standard procedures.

Magnetic moments were obtained via the Evans method with use of coaxial NMR tubes (Wilmad Glass) on a JEOL 100 MHz NMR spectrometer.[12] Spectra were recorded at 25 °C in $H_2O$ with 2% *tert*-butyl alcohol as the reference peak.

ESR spectra were obtained at 77 K, with use of a quartz finger dewar, on a Varian E-4 spectrometer operating at 9.2 GHz. Samples were run in quartz tubes in a 5:1 (v/v) glycerine–water glass. DPPH was used as a standard. EPR parameters for the CuEHPG complexes were obtained by spectral simulation using a program, EPRPOW, originally written by Belford and White and subsequently modified by Chasteen.[13]

Electochemical measurements were performed in water as previously described, with use of a hanging mercury drop electrode and 1 M $KNO_3$ as a supporting electrolyte.[14]

## Results and Discussion

**Ligand.** Ethylenebis((o-hydroxyphenyl)glycine) (EHPG) as commercially available is reported to consist of an unspecified mixture of meso and *rac* isomers. Since in the course of this study we have prepared metal complexes containing either the pure meso or *rac* ligand we have been able to identify both isomers of the ligand by NMR. The NMR data for the pure meso, pure *rac*, and commercial EHPG samples in alkaline $D_2O$ are shown in Table I. The two isomers are easily distinguished by the position of the sharp singlet of the α-H at 4.19 ppm for the *rac* isomer and 4.25 ppm for the meso. The commercial ligand displays two singlets at these positions, of equal intensity, which corresponds to a 1:1 mixture of the two isomers. The protons of the ethylenediamine chains are observed as symetrical $A_2B_2$ patterns centered at 2.63 ppm for both isomers. The existence of this $A_2B_2$ pattern rather than a singlet indicates that the ethylenediamine chain is locked into a particular conformation even in the absence of a metal ion.

This is a contrast to what is found in the related ligands, EDTA and EDDA, where only a singlet is observed for these protons, indicating free motion of the ethylenic carbons.[15,16]
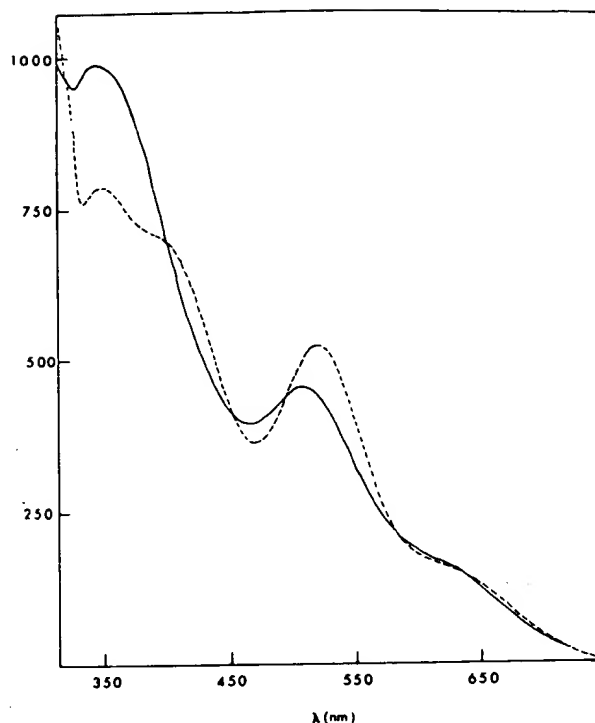


**Figure 1.** Optical spectra of *meso*- (—) and *rac*-Co$^{III}$EHPG (---) in water.

We attribute this difference to strong internal hydrogen bonding in the deprotonated ligand as described by Martell.[17]

**CoEHPG⁻.** The structure of the cobalt(II) salt of *rac*-CoEHPC has recently been determined by single-crystal X-ray diffraction.[6] As expected, the *rac* isomer has the six-membered phenolate rings in the equatorial positions and the five-membered carboxylate rings in axial sites.[18] The meso isomer, which has been examined only in solution, presumably has the geometry of *meso*-FeEHPG, which is fixed by the ligand, i.e., one axial and one equatorial phenolate.[19]

The optical spectra of the two isomers are shown in Figure 1. Low-spin $d^6$ cobalt(III) complexes normally display two visible bands ostensibly associated with the $^1A_1 \rightarrow {}^1T_1$ and $^1A_1 \rightarrow {}^1T_2$ transitions in octahedral crystal fields. Both complexes show a splitting of the lower energy visible band, which is indicative of less than cubic symmetry. We can treat these complexes as being tetragonally distorted octahedra, having pseudo-$D_{4h}$ symmetry, with the equatorial plane being that containing the ethylenediamine nitrogens and the quasi-$C_4$ axis perpendicular to it. We can then utilize the procedure of Wentworth and Piper to analyze the spectra.[20] For the meso complex the equatorial field will be the average of one carboxylate, one phenolate, and two amine donors, while for the *rac* complex, the field will be the average of two amine and two phenolate donors. By the Wentworth and Piper analysis the low-energy band, denoted band I, is expected to contain two components. The energy of the lower wavelength component of these is a direct measure of the equatorial ligand field, provided the equatorial donor atoms are higher in the spectrochemical series than the axial. Indeed, from Figure 1, band I does consist of two components in both complexes and the equatorial field contains two amino nitrogens while

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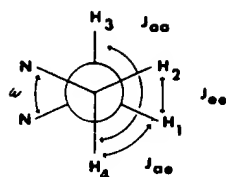
**Figure 2.** Assignment of the coupling constants for the ethylenediamine moiety of CoEHPG.

the axial donors are either carboxylate or phenolate oxygens. Calculation of $Dq_{eq}$ for the meso and *rac* complexes gives 2337 and 2290 cm$^{-1}$, respectively.

A second d–d band is also expected in the visible region, which would correspond to a $^1A_1 \rightarrow {}^1B_2$ transition in tetragonal symmetry. In *rac*-CoEHPG two bands are found in the 300–400-nm region, one with $\lambda = 395$ nm, $\epsilon = 570$ L/(mol cm), and a second with $\lambda = 350$ nm, $\epsilon = 633$ L/(mol cm). Such a splitting might be expected, on the basis of the lifting of the degeneracy of the octahedral $^1T_2$ component into $^1E$ and $^1B_2$ states in a tetragonal field. However, no splitting of this so-called band II has been observed in any tetragonally distorted $O_h$ cobalt complex. Therefore we are inclined to assign the band at 350 nm as a rather weak charge-transfer transition, such as that seen in the complexes of EHPG with other easily reducible metals, although this assignment has to remain tentative. The meso isomer shows only a single asymmetric peak at ca. 348 nm with $\epsilon \simeq 1000$ L/(mol cm), which we assign as an overlap of the charge-transfer band with band II.

The redox potentials for the two isomers of CoEHPG have been measured by cyclic voltammetry. On a mercury drop electrode at neutral pH, *rac*-CoEHPG undergoes a nearly reversible one-electron reduction as determined by a peak to peak separation of nearly 60 mV and a ratio of $i_c/i_a \simeq 1$. The potential for this process is –270 mV vs. SCE. The meso isomer is somewhat more difficult to reduce with an $E_{1/2}$ of –308 mV vs. SCE.

The diamagnetic cobalt complex has allowed a comparison of the solid-state structure with that in solution, as determined by high-resolution NMR. The NMR spectra of the *rac* isomer consists of two sets of multiplets for the phenyl protons centered at 7.0 and 6.4 ppm (8 H), two quasi-quartets at 3.26 and 2.57 ppm (4 H), and a singlet at 3.97 ppm (2 H), which is assigned as the $\alpha$-H's on the chiral carbons. Only a single peak is expected for the two $\alpha$-H's in the *rac* isomer, as they are magnetically equivalent. The two deceptively simple four-line patterns centered at 2.57 and 3.26 ppm represent an $A_2B_2$ system, indicating a gauche conformation of the ethylene diamine portion of the EHPG ligand. An analysis of the spectra indicated two sets of coupling constants that would reproduce the spectra:

| | |
|---|---|
| $J_{12} = +0.0$ | $J_{12} = +0.0$ |
| $J_{13} = +6.0$ | $J_{13} = -12.0$ |
| $J_{14} = -12.0$ | $J_{14} = +6.0$ |
| $J_{34} = +15.0$ | $J_{34} = +15.0$ |

These sets differ only in the arbitrary labeling of nuclei 3 and 4 and in the sign of the coupling constants. The second set was chosen, as the observed coupling of $J_{13}$ of –12.0 Hz is that normally expected for geminal pairs.[21] Thus the complete assignment of coupling constants is as indicated in Figure 2 (Hz): $J_{gem} = -12.0$, $J_{aa} = 15.0$, $J_{ae} = 6.0$, $J_{ee} = 0.0$. The axial protons, 3 and 4, are found 0.69 ppm higher than the equatorial protons, an assignment that is consistent with those in some completely organic systems.[22]
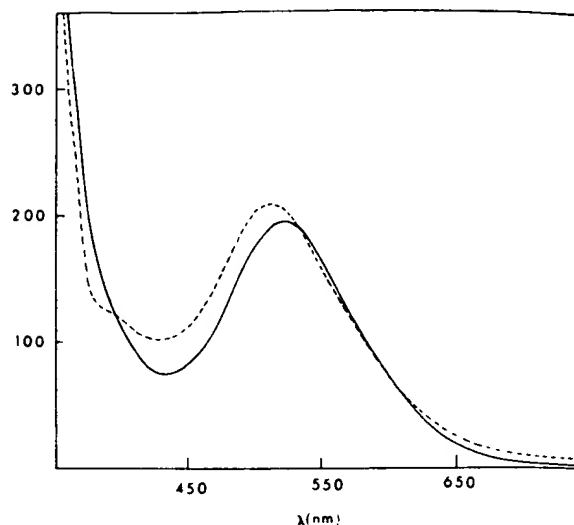


**Figure 3.** Optical spectra of *meso-* (- - -) and *rac*-Cr$^{III}$EHPG (—) in water.

From this analysis it is possible to predict the dihedral angle, $\omega$, between the $N_1$–C–C and $N_2$–C–C planes from the Karplus equation.[23] Although it is dangerous to use this relationship indiscriminately, it has been found to relate the dihedral angle between two coupled protons to their coupling constant:

$$J_{ab} = 8.5 \cos^2 \phi_{ab} - 0.28$$

The observation of a coupling constant of 0.0 Hz for the equatorial–equatorial coupling indicated that the angle between these protons is ~90°. The equatorial–axial interaction predicts a dihedral angle ($\omega$) of 30°. This is to be compared to the 29.3° angle found in the solid state, indicating a retention of the gauche structure in solution. As noted earlier, *rac*-Co(EHPG)$^-$ may exist as one of eight possible isomers consisting of four diasteromeric pairs of enantiomers.[6] Two of these pairs are sterically unlikely as they have two six-membered chelate rings in axial positions. The remaining two pairs, $\Lambda SS\lambda$ ($\Delta RR\delta$) and $\Lambda SS\delta$ ($\Delta RR\lambda$), cannot be distinguished by the NMR spectrum.

The NMR of the meso isomer displays complex multiplets centered at 6.90 and 6.49 ppm (8 H) for the phenyl protons and two sharp singlets at 4.1 and 3.7 ppm (1 H each) for the protons on the chiral carbons, which are now inequivalent. The ethylenediamine portion of the spectrum shows a doublet at 3.1 ppm (1 H) and a broad singlet at 2.6 ppm (3 H). From spin-decoupling experiments it has been determined that these two peaks are spin coupled to each other and thus do not represent two different conformations in solution in a 3:1 ratio. The observation that three of the ethylenediamine protons coincidentally have the same chemical shifts would imply that three of the protons are about equally affected by the diamagnetic anisotropy of the C–N bonds with the fourth proton being more deshielded. This is consistent with the asymmetric skew or "envelope" conformation found in the *meso*-FeEHPG complex via X-ray crystallography.[19] Thus the meso ligand appears to adopt an ethylenediamine ring conformation different from that of the *rac*, both in solution and in the solid state.

**CrEHPG$^-$.** For d$^3$ chromium complexes in octahedral or near-octahedral symmetry two crystal field bands are expected. These bands correspond to the $^4A_2 \rightarrow {}^4T_2$ and $^4A_2 \rightarrow {}^4T_1$ transitions. The crystal field splitting, $10Dq$, can be taken directly from the wavenumber of the $^4A_2 \rightarrow {}^4T_2$ transition and

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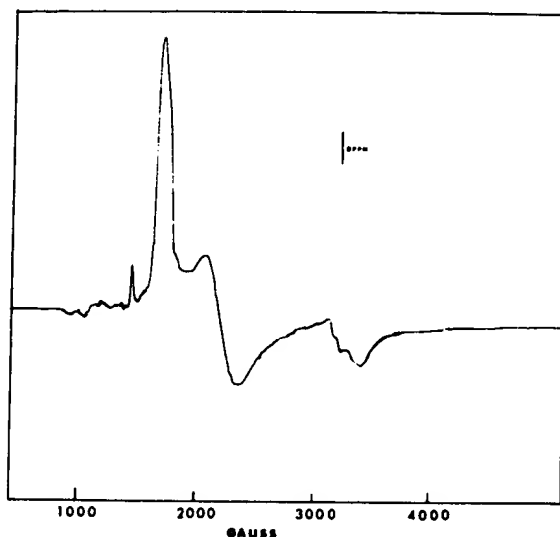
**Figure 4.** EPR spectrum of *rac*-CrEHPG in 5:1 glycerine–water glass at 77 K at 9.14 GHz. Instrument parameters: sweep width 4000 G, modulation amplitude 10 G, gain 8 × 10⁴, sweep time 8 min.

the ratio $\nu_2/\nu_1$ can be used to calculate the Racah parameter, $B$.[24] The visible spectra of the two isomers of Cr$^{III}$EHPG are shown in Figure 3. The *rac* isomer has a band at 511 nm, $\epsilon = 207$ L/(mol cm), and a shoulder at ~390 nm, which can be assigned as the $^4A_2 \rightarrow {}^4T_2$ and $^4A_2 \rightarrow {}^4T_1$ transitions, respectively. This leads to values for $10Dq$ and $B$ of 19570 and 642 cm$^{-1}$. The meso isomer has the low wavelength band shifted to 523 nm with $\epsilon = 180$ L/(mol cm). The second band is almost totally obscured by the tailing of the intense ligand $\pi-\pi^*$ transitions into the near-UV, thus preventing calculation of "$B$". The value of $10Dq$ calculated is 19120 cm$^{-1}$. Neither of the isomers of CrEHPG displays anything assignable as a charge-transfer transition in the visible region. This is not unexpected, given the difficulty in reducing Cr(III) (vide infra).

No redox processes were evident for the CrEHPG over the range of +1.0 to −1.0 V in H$_2$O on mercury, carbon, or Pt electrodes. This is an unsurprising result, given the difficulty in reducing even the hexaaquochromium(III) ion and the propensity for the EHPG ligand to stabilize the trivalent state.

The EPR spectrum of CrEHPG$^-$ has also been recorded. The spectra of the *meso*-CrEHPG in a glycerine–water glass at 77 K is shown in Figure 4. Although the spectrum has not been analyzed in detail, it does display the characteristic features of chromium(III) in a rhombic or nearly rhombic electronic environment with resonances at $g' = 3.71, 2.76$, and 1.91.

**MnEHPG$^-$.** The Mn(III) complex of EHPG has not been previously reported. We find that it can be prepared, albeit in low yield, either from the divalent metal salt followed by air oxidation or directly from discreet Mn(III) compounds. Frost and Martell noted that at high pH it was impossible to titrate Mn(II) solutions of EHPG due to irreversible oxidation.[17] They noted this oxidation even under inert atmosphere and concluded that the oxidation occurs at the expense of the solvent or ligand. The low yields of Mn$^{III}$EHPG are attributable to this ligand reduction when Mn(II) salts were used and to ligand oxidation when Mn(III) compounds were employed. This is an additional example of the tremendous ability of the EHPG ligand to stabilize the trivalent over the divalent state of many transition metals. It is also evident from the change in redox potential from highly positive voltages for the aquoiron and -cobalt ion$^{3+}/{}^{2+}$ couples to relatively negative

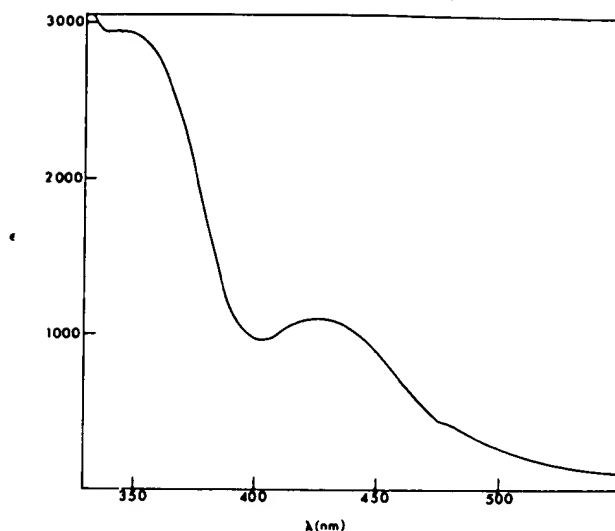(24) Lever, A. B. P. *J. Chem. Educ.* **1968**, *11*, 711.



**Figure 5.** Optical spectrum of *rac*-Mn$^{III}$EHPG in water.

voltages for their EHPG complexes.

The small amount of the Mn(III) complex that was isolated crystallized from aqueous ethanol solutions as thin hexagonal plates. Unfortunately these crystals were not suitable for X-ray structural analysis. The presence of trivalent manganese was confirmed by magnetic susceptibility. A value of $\mu_{eff} = 5.1$ $\mu_B$ was found, close to the spin-only value of 4.9 $\mu_B$ expected for the d$^4$ Mn(III) ion.

The optical spectra of MnEHPG$^-$ were dominated by a moderately intense charge-transfer transition. These brown solutions had $\lambda_{max} = 427$ nm, $\epsilon = 1175$ L/(mol cm), for the *rac* complex and $\lambda_{max} = 432$ nm, $\epsilon = 1100$ L/(mol cm) for the meso derivative. The spectra also displayed a shoulder at 480 nm, $\epsilon = 570$ L/(mol cm), and an additional band at 350 nm, $\epsilon \simeq 3000$ L/(mol cm). A spectrum of the *rac* isomer is shown in Figure 5. The band at 480 nm is assigned as the d–d, $^5E_g \rightarrow {}^5T_{2g}$ transition, although treatment of a Mn(III) complex as octahedral or pseudooctahedral is probably dubious, due to the expected Jahn–Teller distortion of the degenerate $^5E_g$ ground state.

The *rac*-MnEHPG complex is readily reducible on the mercury drop electrode at a potential of −240 mV vs. SCE. However, this behavior is only observed at high pH (pH 11–12), where a nearly reversible (peak to peak separation 62 mV, $i_c/i_a \simeq 1$) one-electron reduction occurs. At lower pH only irreversible waves are observed.

**CuEHPG$^{2-}$.** Copper EHPG has been studied in solution by Frost and Martell.[17] At neutral pH or below, a typical blue Cu$^{2+}$ complex is observed, which no doubt involves only coordination to the ethylenediamine nitrogens and the carboxylate oxygens. However, as the pH is raised, a new green complex is formed, which involves coordination by the phenol groups. Recent crystallographic analysis confirms a six-coordinate complex with bound phenols, similar to those of the other metals but subject to a severe Jahn–Teller distortion.[6] As is usually the case, the axial bonds are found to be substantially lengthened relative to those in the equatorial plane (2.42 vs. 1.94 Å). In the *rac* isomer whose structure was determined, this results in two very long copper–carboxylate bonds. However, in the meso isomer, this presumably results in one long carboxylate and one long phenolate bond.

The optical spectra at pH 10.0 of *meso-* and *rac*-CuEHPG are shown in Figure 6. Both display a single weak band at ~650 nm assigned as a d–d transition and a more intense band at ~365 nm, which is either a L $\rightarrow$ M or M $\rightarrow$ L charge-transfer transition. It was expected that this charge-transfer band would be very sensitive to the geometry (axial or equatorial) of the phenolate group, as the position of CT bands is
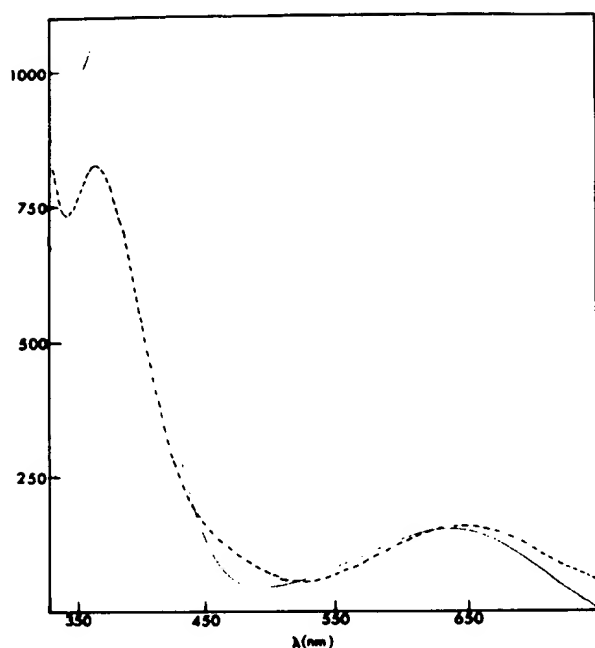
**Figure 6.** Optical spectra of *meso-* (—) and *rac*-Cu$^{II}$EHPG (- - -) in water at pH 10.0.

in part dependent on internuclear separation, and crystal structures revealed that the axial bonds are substantially lengthened. However, while the d–d band shifts from 650 nm in the *rac* complex to 630 nm in the meso, the charge-transfer band shifts only 8 nm ($\sim$0.6 × $10^3$ cm$^{-1}$), from 370 to 378 nm. This shift is not significantly larger for the copper complexes than for the iron (0.58 vs. 0.48 × $10^3$ cm$^{-1}$). While placing a phenolate group and its appended six-membered chelate ring in an axial position is seen to shift the L → M charge-transfer bands to lower energy for the Fe$^{III}$- and Mn$^{III}$EHPG complexes, the Jahn–Teller lengthening of the axial bands in the copper complex would probably shift this band in the opposite direction due to a decreasing metal–phenolate overlap. The overall result might be only a small shift, as is observed. Alternatively the $\sim$370-nm band in the copper complex could be assigned as a M → L charge transfer rather than L → M, and indeed this assignment has already been given for the charge-transfer band in a series of related copper phenolate complexes.[25] Interestingly, the EPR spectra of the CuEHPG complexes are also not very sensitive to geometry. The EPR spectrum of *rac*-CuEHPG in a glycerine–water glass at 77 K is shown in Figure 7. The meso complex exhibits a virtually identical spectrum. This is in comparison to the situation found for the *meso-* and *rac*-FeEHPG complexes. In these complexes the EPR is quite sensitive to the differing geometries of the two isomers, and they are easily distinguished by this technique.[7] Analysis of the CuEHPG spectrum is consistent with a rhombic electronic environment and gives the EPR parameters indicated in Table II. No nitrogen superhyperfine splitting is observable. However, the rather broad line widths ($\sim$25 G) of the $g_z$ component needed to fit the spectrum are consistent with some unresolved Cu–N interaction.

## Comparison to Transferrin

There has been considerable recent controversy as to the number and precise geometry of the donor groups in transferrin. Some have favored a model that calls for three tyrosines, two histidines, water, and bicarbonate ion as ligands while others prefer a two-tyrosine, two-histidine, bicarbonate ion, and hydroxide ion model.[5,26] The main evidence for each
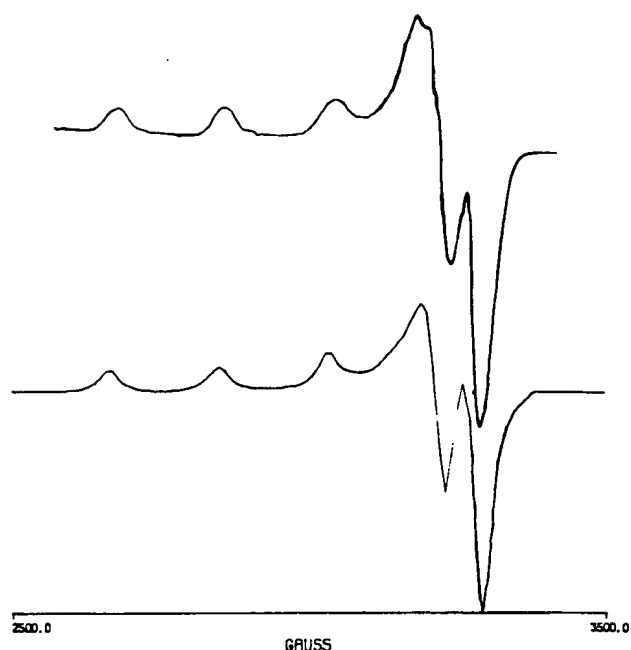
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**Figure 7.** EPR spectra of *rac*-CuEHPG in 5:1 glycerine–water glass at 77 K at 9.14 GHz. Upper curve is the experimental spectrum obtained with 1000-G sweep width, 4-G modulation amplitude, 4 × $10^4$ receiver gain, and 8-min sweep time. The lower curve is the spectrum simulated with the parameters shown in Table II.

**Table II.** Comparison of Optical and EPR Spectra of Metallo–EHPG and –Transferrin Complexes

| metal | trans-ferrin[a] | lactoferrin[b] | *rac*-EHPG | *meso*-EHPG |
|---|---|---|---|---|
| Co(III) | 405 | 405 (5170) | 350 (633) | 348 |
| Mn(III) | 430 | 435 (4810) | 427 (1175) | 432 (1100) |
| | 330 | 340 (4975) | 350 ($\sim$3000) | 350 ($\sim$3000) |
| Cr(III) | 440 | 442 (260) | 390 (sh) | |
| | 615 | 612 (140) | 511 (207) | 523 (180) |
| Fe(III) | 470 | 465 (2070) | 475 (4000)[c] | 485 (4000) |
| Cu(II) | 440 | 438 (2400) | 370 ($\sim$830) | 378 ($\sim$1080) |
| | 650 | 677 (310) | 650 ($\sim$175) | 630 ($\sim$167) |

| complex | $g'_x$ | $g'_y$ | $g'_z$ | $A_z$, G |
|---|---|---|---|---|
| **Cr(III)** | | | | |
| *rac*-EHPG | 2.76 | 1.91 | 3.71 | |
| *meso*-EHPG | ... | ... | ... | |
| transferrin[a] | $\sim$2.3 | 1.98 | 5.49 | |
| **Fe(III)** | | | | |
| *rac*-EHPG[c] | 4.30 | 4.24 | 4.38 | |
| *meso*-EHPG[c] | 4.31 | 4.24 | 4.41 | |
| transferrin[d] | 4.27 | 4.08 | 4.39 | |
| **Cu(II)** | | | | |
| *rac*-EHPG | 2.026 | 2.028 | 2.212 | 184 |
| *meso*-EHPG | 2.026 | 2.028 | 2.212 | 184 |
| transferrin[e] | 2.042 | 2.059 | 2.312 | 156 |

[a] From ref 28. [b] From ref 29. [c] From ref 7. [d] From ref 5. [e] From ref 30.

of these views is given in the quoted references. A recent study comparing a variety of iron-containing model compounds with transferrin has appeared.[27] The authors conclude that the three-tyrosine model is to be considered preferable on the basis

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Table III. Ligand Field Parameters for Chromium Complexes of EHPG and Transferrins

| complex | $10Dq$, cm$^{-1}$ × 10$^3$ | $B$, cm$^{-1}$ |
|---------|---------|---------|
| CrTr | 16.26 | 650 |
| CrLf | 16.34 | 628 |
| CrEHPG | 19.57 | 642 |
| CrEHPG | 19.12 | |

of an evaluation of optical spectra. Unfortunately, there are a large number of iron complexes with moderately intense bands in the same region (~470 nm) as transferrin. Among them are complexes of the catechols, hydroxamates, simple phenols, and thiocyanate as well as EHPG. The plethora of iron complexes exhibiting a charge-transfer band in this region makes it difficult to substantiate any conclusions based solely on the optical spectra of these models. However, much more useful information about transferrin can be gained by extending these comparisons to metal ions other than iron.

The rather good qualitative if not quantitative similarity in the optical spectra of the Cr, Co, Mn, Cu, and Fe complexes of EHPG with their corresponding transferrin analogues seems to indicate that the donor groups in EHPG and transferrin are in fact quite similar. The spectra of the chromium complexes may be particularly informative as the lack of a CT band in the visible region allows the observation of the ligand field bands. For a d$^3$ ion such as Cr$^{3+}$ in an octahedral or near-octahedral field, calculation of $10Dq$ is a trivial exercise. In addition, as long as at least two of the three expected transitions are observable, we may calculate values of $B$ (the Racah parameter) rather accurately using tables such as those compiled by Lever.[24] These data are shown in Table III. The absorption peak maxima of transferrin and lactoferrin seem inconsistent with an N$_2$O$_4$ donor set. This question has been considered by Ainscough et al. They conclude that the donor set for CrTr or CrLf may be a N$_2$O$_3$Cl type, where a chloride is still bound to the chromium. Although our data would be consistent with this view, it is difficult to imagine the chromium ion being sufficiently inert that no aquation would occur over several weeks. In view of the sensitivity of the d–d bands in CrEHPG to isomer type, an alternative possibility would be to have two axial tyrosines, with somewhat lower p$K_a$'s than those found in EHPG, in the transferrin complex. This combination would have the effect of shifting the absorption

maxima to longer wavelength.

While the spectra of the metal EHPG complexes display all the characteristic features of the transferrin spectra, they are often shifted in energy or intensity (Table II). The *rac* iron and manganese complexes of EHPG display visible spectra that are nearly identical in band positions with those of their transferrin analogues, while the spectra of the cobalt, copper, and chromium complexes of EHPG are shifted to higher energy. The reasons for these differences may have several origins: The first is the negative charge on the EHPG complexes. This is expected to have the effect of increasing the energy of LMCT transitions. The second possibility is the steric strain associated with the five-membered carboxylate chelate rings. As has been pointed out by Martell, EHPG with its five-membered carboxylate chelate rings is strained upon metal binding. The position (axial or equatorial) of the strained ring system is seen to affect the optical spectra as demonstrated by the spectral differences between the meso and *rac* isomers. The third reason may be electronic effects and p$K_a$ values. There appears to be evidence that the p$K_a$'s of the tyrosines in conalbumin, at least, are unusually low with values of ~8.5 and 10.5 reported.[31] NMR work has shown that the histidine imidazoles have unusually high p$K_a$'s: ~7.5 and 7.6.[32] The dissociation constants for EHPG are 6.3 and 8.6 for the amino nitrogens and 10.2 and 11.7 for the phenols.

Work in modifying the basic structure of EHPG to examine the electronic and steric effects discussed is under way, and it appears hopeful that some derivative of EHPG will be a very useful model in probing the binding site and mode of action of the transferrins.

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# Origin of the Red Color in Copper(II)-Doped Ethylenediammonium Tetrachloromanganate(II)

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Crystals of the two-dimensional antiferromagnets A$_2$MnCl$_4$ (A = RNH$_3$ or A$_2$ = R(NH$_3$)$_2$) doped with small amounts of copper(II) are deep red. The origin of the phenomenon is investigated by single-crystal absorption spectroscopy of a variety of copper(II)-doped manganese(II) compounds. An intense absorption band centered at 20800 cm$^{-1}$ and completely polarized in the plane of the two-dimensional lattice is responsible for the color. The band is interpreted as a Cl → Cu electron-transfer transition in a CuCl$_6$ center with a "strained" coordination.

## Introduction

Salts of the layer perovskite family, (RNH$_3$)$_2$MX$_4$, have been intensely studied during the past decade for a variety of reasons. They consist of layers of corner-sharing octahedra

(or pseudooctahedra) sandwiched between layers of the monosubstituted ammonium ions.[1] When the metal ion is a paramagnetic center such as copper(II), manganese(II), or iron(II), the salts provide excellent realizations of two-di-

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